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Effect of Desiccation, pH, Heat, and Ultraviolet Irradiation on Viability of *Baculovirus penaei*

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Effect of Desiccation, pH, Heat, and Ultraviolet Irradiation on Viability of *Baculovirus penaei*

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Abstract

Tolerance of *Baculovirus penaei* virus to desiccation, pH, heat, and ultraviolet irradiation (uv), as well as its survival in 32 ppt sea water, was investigated using larval *Penaeus vannamei* in a bioassay. Test shrimp were sampled from 36 to 264 hr after food-borne exposure to treated *B. penaei* and examined for patent infections (polyhedral inclusion bodies detectable in fresh preparations of hepatopancreas) with light microscopy. *B. penaei* was completely inactivated within 30 min after exposure to pH 3, whereas exposure to pH 11 extended the prepatency period (period before infection is apparent) but did not inactivate the virus. *B. penaei* was inactivated by 10-min exposures to temperatures of 60–90°C. Ultraviolet irradiation for 40 min at a wavelength of 254 nm when *B. penaei* was 5 cm from the light source inactivated the virus (measured as a cumulative uv dosage of 7.08×10^6 $\mu W \cdot sec/cm^2$). Desiccation for 48 hr also inactivated the virus, and that method is the most practical to halt or prevent infections in some aquaculture facilities. *B. penaei* free from its host survived 32 ppt sea water at 22°C for 7 days and at 5°C for at least 14 days.

Keywords: *Baculovirus penaeid*, *Penaeus vannamei*, nuclear polyhedrosis virus, pH, ultraviolet, heat, desiccation, viability

Introduction

Infections and disease caused by *Baculovirus penaei* in both cultured and wild penaeid shrimps are influenced by environmental parameters. Consequently, manipulation of these param-

eters can control or eliminate infections, benefiting production of cultured penaeids. *B. penaei* causes disease in both cultured and wild penaeid shrimps along the coasts of the Gulf of Mexico, Central America, and South America. The virus can be introduced into a culture system by contaminated penaeid shrimps, water, personnel, and equipment. Failure to adequately disinfect those sources allows transmission of virus within and among shrimp facilities, resulting in economic losses from mortalities and from transportation restrictions. When BP epizootics occur in hatcheries, they typically are acute, causing larval and postlarval mortalities approaching 100% (e.g., Lightner, 1988).

Since the original description of *B. penaei* in *Penaeus duorarum* from Florida (Couch, 1974), the virus has been reported from *P. aztecus* and *P. setiferus* along the northern Gulf of Mexico (Overstreet and Howse, 1977; Couch, 1981) and from *P. vannamei*, *P. stylirostris*, *P. penicillatus*, *P. schmitti*, *P. paulensis*, and *P. subtilus* in culture facilities or the coastal waters of the Pacific Ocean in Central and South America (Lightner and Redman, in press).

Researchers have examined the effects of chemical and physical conditions on the infectivity of terrestrial insect viruses. Ultraviolet (uv) light inactivated or reduced infectivity of *Trichoplusia ni* nuclear polyhedrosis virus (NPV) (Jaques, 1967), *Heliothis* NPV (Bullock et al., 1970), and *Neodiprion swainei* NPV (Smirnoff, 1972). Extreme pH conditions of 1.2 and 12.4 reduced infectivity of *Heliothis* NPV (Ignoffo and Garcia, 1966), and heat inactivated cytoplasmic-polyhedrosis viruses of *Bombyx mori* (see Aruga et al., 1963) and *Colias eurytheme* (see Tanada and Chang, 1968). Gudauskas and Canerday (1968) further demonstrated reduced infectivity of *Heliothis* NPV and *T. ni* NPV when exposed to extreme acid or alkaline conditions, uv light, and heat.

In contrast, little information is available about the effects of chemical and physical conditions on the infectivity of aquatic shrimp baculoviruses. Momoyama (1989a) inactivated baculoviral midgut gland necrosis (BMN) virus using uv light, heat, and desiccation. Later papers by the same author demonstrated the tolerance of BMN to ether, NaCl, pH, freezing conditions, and sea water when free from its host (Momoyama, 1989b, 1989c). BMN, unlike *B. penaei*, is a nonoccluded baculovirus; it infects *P. japonicus*. We investigated several parameters to determine their effect on infectivity of *B. penaei:* heat, uv light, extreme acid and alkaline conditions, desiccation, and 32 parts per thousand (ppt) sea water.

Materials and Methods

The virus strain used was originally collected in Ecuador by James Brock (Hawaii Department of Land and Natural Resources) from a combination of wild broodstock and pond-reared juveniles of *P. vannamei*. We at the Gulf Coast Research Laboratory used that material to amass large numbers of infected larvae of *P. vannamei* for these and other studies (Overstreet et al., 1988). Aliquots of approximately 200 frozen infected larvae with an addition of 1 ml of 32 ppt sea water (produced from hw-Marinemix synthetic sea salts) were used for each experimental group. Each aliquot of infected larvae was thawed, homogenized with a Dounce tissue grinder or razor blade, and treated according to one of the methods described later in this section. Treated viral homogenate was then fed one time over a 2-hr period to 150 ml of a culture of the rotifer *Brachionus plicatilis* as a carrier to facilitate delivery of virus to the shrimp larvae (Overstreet et al., 1988). The fed rotifers and

residual uneaten viral material were mixed and divided into three 50-ml portions, and one of those portions was introduced into each of two or three replicate, aerated, 1-liter Imhoff cones (each cone contained 125 virus-free second or third substage protozoeal larvae of *P. vannamei*). Water contaminated with uneaten rotifers and virus was poured through 380 or 500 μm-Nytex-meshed sieves 36–48 hr after the virus was introduced. This process retained the larvae which were washed before being reintroduced into rinsed cones with uncontaminated water.

To avoid contamination among experimental groups and among replicates, we carefully discarded the contaminated water and sanitized the sieves, forceps, pipettes, and hands with an excessively strong solution of Wescodyne (>500 ppm, a detergent containing at least 1.6% titratable iodine) after each water change. Water was changed every 2 days to prevent an accumulation of waste products and bacteria. Two or three replicate cones were maintained for each group to ensure sufficient numbers of shrimp to sample and to detect accidental contamination. Shrimp were maintained on a diet of the brine shrimp *Artemia franciscana*.

Negative controls for each experiment were maintained in the same manner as their experimental counterparts, except that no virus was added. Overstreet et al. (1988) provided a detailed description of the bioassay design and methodology.

Diagnosis of *B. penaei* was made on the basis of microscopic observation of polyhedra in fresh whole mounts of shrimp hepatopancreas. The hepatopancreas was re moved intact with two fine forceps on a 51 by 76-mm glass microscope slide under a dissecting microscope (Overstreet et al., 1988). The tissue was then gently covered. with a coverslip and examined with light microscopy for the presence of polyhedra.

Sampling was conducted at various time intervals for each experimental group until the prevalence of infection reached 95 to 100% or remained at zero for 4 days or more after the assay began. In most cases, we randomly sampled six to nine living larvae from each replicate during routine water changes. In cases where excessive mortalities occurred, sampling from those groups or replicates was eliminated, postponed, or reduced. Also, not all of the sampling times in different tests corresponded because of time constraints involving hatching times. Fisher's exact test was used to determine whether results from replicate samples could be pooled and whether pooled data were homogeneous.

B. penaei ***suspended in 32 ppt sea water***

Aliquots of homogenate were pipetted into 29 ml of 32 ppt sea water 79, 59, 14, or 7 days before the assay. Each of the above aliquots was inoculated once a week with 0.3 ml of antibiotic (5 ppm of both streptomycin and penicillin) and was held at 22°C. Additional virus aliquots were held in sea water for 59 and 14 days at 5°C, with one of those administered antibiotics.

Desiccation

Aliquots of homogenized infected larvae without added sea water were placed in the top portion of 5.5-cm glass Petri dishes, partially covered with Parafilm, and stored at 22°C for 12, 24, or 48 before the assay. Based on visual observations, aliquots appeared slightly moist at 12 hr and completely dry at 24 and 48 hr.

pH

Aliquots of homogenized infected larvae were pipetted into 9 ml of 0.2 M sodium phosphate buffer at pH values of 3, 7, 11 or unbuffered sea water at pH 8.8. The pH of buffers was established by mixing appropriate amounts of 1 N NaOH with NA_2HPO_4 or 1 N HCl with NaH_2PO_4. Viral homogenate-buffer mixtures as well as unbuffered virus in sea water were incubated at room temperature (22°C). Mixtures of virus buffered at pH 7 and 11 as well as that suspended in pH 8.8 sea water were incubated for either 2 or 6 hr. Mixtures of virus buffered at pH 3 were incubated for either 30 min or 2 hr. After the incubation periods, samples were centrifuged for 25 min at 5000*g*, supernatant was removed, and precipitate was rehydrated with 32 ppt sea water before being assayed.

Heat

Aliquots of homogenate in 4 ml of 32 ppt sea water were pipetted into thin-walled, 20-ml glass tubes. The tubes were maintained in water baths at constant temperatures of 50, 60, 70, 80, or 90°C for 10 min, removed and placed in tap water, and cooled to room temperature; the contents were then assayed.

Ultraviolet light

Aliquots of homogenized larvae in 4 ml of 32 ppt sea water were pipetted into the bottoms of 9-cm glass Petri dishes and irradiated with light from 6-W Cole-Parmer 9815-series uv lamps. Material was irradiated for various times at wave lengths of 365 or 254 nm, at a distance 5 cm from the light source. Light intensities were measured with Spectroline DM-365x and DM-254x ultraviolet meters as 1.64 and 2.95×10^3 $\mu W/cm^2$, respectively.

Results

B. penaei ***suspended in 32 ppt sea water***

Both the temperature at which occluded and nonoccluded virions liberated from host cells were maintained and the length of time after which virions were liberated from their host affected the prevalence of subsequent *B. penaei* infections (Table 1). Viral homogenate suspended in sea water for 7 days at 22°C remained virulent, but at 36 hr it produced a lower prevalence of infection than controls fed virus on the day it was thawed. Suspensions of virus kept at 22°C for longer periods of 14, 59, and 79 days, however, became completely inactivated. In contrast, virus held at 5°C for 14 days produced 50–65% prevalence of patent infection at 96 hr, whereas material held 59 days never induced an infection. The combined mixture of streptomycin and penicillin had no effect on the resulting *B. penaei* infections.

Table 1. Viability of *Baculovirus penaei* in 32 ppt Salt Water for Various Periods at Two Temperatures

Virus treatment[a]	Period in 32 ppt (days)	Hr post-exposure[b]	No. of replicates sampled[c]	Infection[d]		Percentage infected (95% confidence limits)[e]
				No. examined	No. infected	
Without antibiotics, 22°C[f]	0	36	2	14	12 A	86 (57–98)
	7	36	2	9	3 B	33 (8–70)
		120	2	9	9 A	100 (66–100)
		168	2	11	11 A	100 (71–100)
	79	36	2	13	0 B	0 (0–25)
		120	2	9	0 B	0 (0–34)
		168	2	10	0 B	0 (0–31)
With antibiotics, 22°C[g]	0	44	3	22	21 A	95 (77-100)
	14	44	3	21	0 B	0 (0–15)
		96	3	20	0 B	0 (0–17)
		168	3	24	0 B	0 (0–14)
	59	44	3	18	0 B	0 (0–19)
		96	2	12	0 B	0 (0–27)
		168	2	14	0 B	0 (0–23)
With antibiotics, 5°C[g]	0	44	3	22	21 A	95 (77–100)
	14	44	3	25	0 B	0 (0–13)
		96	3	20	13 C	95 (41–85)
		168	1	8	6 A C	75 (35–97)
	59	44	3	22	0 B	0 (0–15)
		96	3	22	0 B	0 (0–15)
		168	3	24	0 B	0 (0–15)
Without antibiotics, 5°C[g]	0	44	3	22	21 A	95 (77–100)
	14	44	2	11	0 B	0 (0–29)
		96	3	22	11 C	50 (28–72)
		168	1	2	2 A C	100 (16–100)
	59	44	3	22	0 B	0 (0–15)
		96	3	21	0 B	0 (0–15)
		168	3	22	0 B	0 (0–15)

a. Free and occluded *B. penaei* virions liberated from approximately 200 homogenized infected *P. vannamei* larvae.
b. Number of hours after assay began.
c. Larvae per replicate = 125.
d. Based on examination of six to nine randomly selected larvae per replicate when available; capital letters in common indicate no significant difference (Fisher's exact test $P \geq 0.05$).
e. From table of confidence limits for percentages.
f. First test.
g. Second test run 6 months after the first, using 5 ppm streptomycin and penicillin as antibiotics.

Desiccation

Viral suspensions desiccated for 48 hr were completely inactivated (Table 2). Desiccation for periods of 12 and 24 hr decreased the prevalences of infection by 69 and 86% (to 26 and 9%), respectively, when examined at 72 hr postinfection; however, by 216 hr, the prevalence of infection for both groups increased to approximately 100%.

Table 2. Inactivation of *Baculovirus penaei* Virus by Desiccation

Desiccation period (hr)[a]	Hr post exposure[b]	No. of replicates sampled[c]	Infection[d]		Percentage infected (95% confidence limits)[e]
			No. examined	No. infected	
0	72	3	19	18 A	95 (74–100)
12	72	3	19	5 B	26 (9–51)
	216	3	24	24 A	100 (86–100)
24	72	3	22	2 B C	9 (l–29)
	216	3	24	23 A	96 (79–100)
48	72	3	21	0 C	0 (0–15)
	96	3	18	0 C	0 (0–19)
	216	2	15	0 C	0 (0–22)

Note: See footnotes *b–e* to Table 1.

a. Desiccation period of free and occluded *B. penaei* virions liberated from approximately 200 infected *P. vannamei* larvae.

pH

Values for infectivity of *B. penaei* following exposure to pH 3, 7, 8.8, and 11 for various time intervals are presented in Table 3. The virus was inactivated within 30 min after exposure to pH 3. Exposing the. virus for 360 min to pH 11 extended the prepatency period of infection in the assayed shrimp by 32 hr when compared to those infected with virus initially exposed to pH 7, 8.8, or 11 for 120 min; however, by 96 hr postinfection, 83% of the shrimp sampled demonstrated infections.

Table 3. Inactivation of *Baculovirus penaei* Virus by Exposure to Various H-Ion Concentrations

pH	Period of exposure (min)[a]	Hr post exposure[b]	No. of replicates sampled[c]	Infection[d]		Percentage infected (95% confidence limits)[e]
				No. examined	No. infected	
3	3	36	2	14	0 B	0 (0–23)
		40	3	20	0 B	0 (0–17)
		72	3	23	0 B	0 (0–14)
		96	2	16	0 B	0 (0–21)
		216	2	14	0 B	0 (0–23)
		264	2	16	0 B	0 (0–21)
3	120	40	3	25	0 B	0 (0–13)
		72	3	23	0 B	0 (0–14)
		96	3	20	0 B	0 (0–17)
		120	3	21	0 B	0 (0–15)
		168	3	23	0 B	0 (0–14)
7	120	40	3	19	19 A	100 (82–100)
8.8[f]	120	40	3	25	24 A	96 (80–100)
11	120	40	3	20	19 A	95 (77–100)
11	360	40	3	21	0 B	0 (0–15)
		72	3	23	9 C	39 (19–59)
		96	3	23	19 A	83 (63–95)
		120	1	6	6 A	100 (54–100)

Note: See footnotes *b-e* to Table 1.

a. Period that free and occluded *B. penaei* virions liberated from approximately 200 homogenized infected *P. vannamei* larvae were exposed to various H-ion concentrations.

f. Virus suspended in unbuffered sea water at pH 8.8.

Heat

B. penaei was completely inactivated by 10-min exposures to temperatures of 60–90°C. Viral suspension maintained at 50°C was just as infective as that maintained at room temperature (Table 4).

Table 4. Inactivation of *Baculovirus penaei* by Heat

Temperature of treatment (°C)[a]	Hr post exposure[b]	No. of replicates sampled[c]	Infection[d]		Percentage infected (95% confidence limits)[e]
			No. examined	No. infected	
20–24	72	3	19	11 A	58 (80–34)
	96	3	23	23 A	100 (86–100)
50	72	3	22	14 A	64 (83–41)
	96	3	29	29 A	100 (88–100)
60	72	3	20	0 B	0 (0–17)
	96	3	22	0 B	0 (0–15)
	144	3	25	0 B	0 (0–13)
70	72	3	24	0 B	0 (0–14)
	96	3	21	0 B	0 (0–15)
	144	3	11	0 B	0 (0–29)
80	72	3	22	0 B	0 (0–15)
	96	3	17	0 B	0 (0–20)
	144	3	25	0 B	0 (0–13)
90	72	3	20	0 B	0 (0–17)
	96	3	21	0 B	0 (0–15)
	144	3	26	0 B	0 (0–13)

Note: See footnotes *b–e* to Table l.

a. Free and occluded *B. penaei* virions liberated from approximately 200 homogenized infected *P. vannamei* larvae and treated for 10 min at listed temperatures.

Ultraviolet light

B. penaei irradiated with uv dosages generated by wavelengths *5* nm remained highly infective; however, a regression analysis (intercept = 117.4, slope = 0.0000145, $r = 0.92$, $P \leq 0.001$, $N = 9$) indicated a linear reduction in the prevalence of *B. penaei* infection in larvae fed virus treated with a wavelength of 254 nm at cumulative uv dosages of $8.85 \times 10^5 - 7.08 \times 10^6$ μW · sec/cm². Lower cumulative dosages of 1.77 and 3.54×10^6 produced by the same 254 nm wavelength resulted in a slightly lower prevalence of infection in samples observed 44 hr postinfection, but prevalence of infection increased to 100% of the larvae sampled at 72 hr. *B. penaei* was completely inactivated by a 40-min exposure to a cumulative uv dosage of 7.08×10^6 μW · sec/cm² generated by the wavelength of 254 nm (Table 5).

Table 5. Inactivation of *Baculovirus penaei* Virus by UV Irradiation

Period of irradiation (min)	Cumulative uv dosage (μW · sec/cm²)[a]	Hr post exposure[b]	No. of replicates sampled[c]	Infection[d]		Percentage infected (95% confidence limits)[e]
				No. examined	No. infected	
0	0	72	2	14	14 A	100 (77–100)
5	4.92×10^5 (365 nm)	72	2	10	10 A	100 (69–100)
	8.85×10^5 (254 nm)	72	2	11	10 A	91 (59–100)
10	9.84×10^5 (365 nm)	44	2	16	16 A	100 (79–100)
	1.77×10^6 (254 nm)	44	2	10	8 A	80 (44–98)
	1.77×10^6 (254 nm)	72	2	10	10 A	100 (69–100)
20	1.96×10^6 (365 nm)	44	2	13	13 A	100 (75–100)
	3.54×10^6 (254 nm)	44	2	11	4 B	36 (11–69)
	3.54×10^6 (254 nm)	72	2	10	10 A	100 (69–100)
40	7.08×10^6 (254 nm)	36	2	13	0 B	0 (0–25)
	7.08×10^6 (254 nm)	48	2	14	0 B	0 (0–23)
	7.08×10^6 (254 nm)	216	2	16	0 B	0 (0–21)
	7.08×10^6 (254 nm)	264	2	16	0 B	0 (0–21)

Note: See footnotes *b–e* to Table 1.

a. Ultraviolet wavelengths and intensity: 365 nm (1.64×10^3 μW/cm²) and 254 nm (2.95×10^3 μW/cm²) when irradiated at a 5-cm distance from free and occluded *B. penaei* virions liberated from approximately 200 homogenized infected *P. vannamei* larvae.

Discussion

Results of this study corroborate those of Momoyama (1989a,b,c) who showed that a virus of penaeid shrimp (BMN) was relatively stable within its seawater environment, just as those from insects can survive in their protected terrestrial habitats, but for much longer periods (Jaques, 1967; Gudauskas and Canerday, 1968). Chemical and physical parameters affecting baculoviruses, however, can be manipulated, and the effects of those changes on viral infectivity differ among at least certain known insect and shrimp viruses.

Our data on longevity of *B. penaei* liberated from its living host cell and those on BMN are similar (Momoyama, 1989a,c). These baculoviruses free in water, trapped in detritus, or located on the dry surface of floors and equipment are capable of transferring diseases within and among aquaculture systems. Both *B. penaei* and BMN remain infective when in

sea water for up to 7 days at water temperatures of 22 and 25°C, respectively, and longer when in colder water. Momoyama (1989a) reported that when nonoccluded BMN was desiccated, complete inactivation occurred within 1.5 hr *B. penaei*, perhaps because a portion of virions is incorporated in polyhedra, produced some infections after contaminated material was desiccated for 12 and 24 hr. However, since desiccation for 48 hr completely inactivated the agent, drying contaminated floors, materials, and fiberglass tanks or raceways for 48 hr or more should provide the shrimp farmer a practical cost-efficient method of disinfecting e culture facilities. Inactivation of a portion of the agents at 24 and fewer hours may reflect defective particles for nonoccluded virus but little or no detrimental effect on occluded ones.

Extreme pH conditions reduce or eliminate infectivity of baculoviruses. Tested insect viruses cannot tolerate alkaline conditions as high as pH 12 or acidic ones as low as pH 2 (Ignoffo and Garcia, 1966; Gudauskas and Canerday, 1968), and our data on *B. penaei* support those of Momoyama (1989b), who demonstrated for a shrimp virus complete inactivation of BMN at pH 3 for 180 min but no effect at pH 10–13 after the same period. Summers (1977) could not solubilize occluded *B. penaei* virions at pH 10.9. The relatively long prepatency period of the infection in the shrimp given *B. penaei* treated at pH 11 for 360 min compared to that treated for 120 min may also reveal selective inactivation of nonoccluded virions. Progressive inactivation of occluded virions could also be a factor.

We exposed a second group of infected tissues to the same four pH conditions using the same methods and examined for polyhedra. We observed polyhedra in all but pH 3 (adjusted with HCl in NaH_2P0_4). The infectivity of that viral material, if still intact, was not pursued. During the initial infection experiment, however, some free defective viral particles were probably not discarded with the supernate. The material was not washed and the organic matter was centrifuged at 5000*g* for 25 min, presumably incorporating some virus in the packed fraction.

Biochemical conditions that effect dissolution of occlusion bodies in shrimp and insect hosts differ. Many insect NPVs are apparently solubilized by high alkaline digestive fluid. For example, the 9.8 pH digestive fluid of *T. ni* or even a pH 9.5 sodium carbonate buffer rapidly dissolved the polyhedral matrix and disrupted the polyhedral envelope of *Autographa californica* (see Pritchett et al., 1984). In contrast, the pH of crustacean digestive systems range from 5 to 7 (Dall and Moriarty, 1983). Preliminary studies in our laboratory indicate the pH of *P. vannamei* and *P. duorarum* digestive tracts from foregut to rectum measure approximately 6–7, and *B. penaei* occlusion bodies were not solubilized after 5- to 10-min exposures to phosphate buffers at pH 4 and 5.5, but they were disassociated within 10–30 sec by a solution of 4 M guanidinium isothiocyanate buffered to a pH of either 5.5 or 10.5. Currently, we do not know if the guanidinium solution will inactivate *B. penaei* virions, but further investigation is planned. This raises a question: how and where are occluded virions released from polyhedra, and how do they infect the HP cells? The mechanisms may involve enzymes triggered by a specific range of pH as assumed for insect species (Granados and Williams, 1986). Our study plus additional unpublished data indicate that occluded virions of *B. penaei* are as stable as insect viruses over a broad range of pH. Tested shrimp and insect NPVs apparently remain highly infective at any pH ranging from 4 to at least 11. Because of *B. penaei* tolerance to a broad range of pH, the disinfection

in aquaculture systems by manipulation of this parameter is probably not economically feasible; however, further study of the role of pH and its effects on *B. penaei* should better define the mechanisms affecting shrimp baculoviral infections.

Heat appears to inactivate viruses of shrimp more readily than those of insects, and this effect probably reflects the corresponding host habitats. The baculovirus *Heliothis* NPV and cytoplasmic polyhedrosis viruses of *Colias eurytheme* and *Bombyx mori* were inactivated by 10-min exposures to 80 and 85°C, respectively (Gudauskas and Canerday, 1968; Tanada and Chang, 1968; Aruga et al., 1963). The lower effective inactivating temperature of 60°C for 10 min for *B. penaei* and 5 min (50°C for 30 min, but partially effective at 35–40°C) for BMN (Momoyama, 1989a) probably reflects the normally protective aspect of an aqueous environment. Except in shallow water during summer months, temperatures seldom get higher than 35°C, and shrimp hosts have difficulty tolerating that stress. On the other hand, heating an aquaculture system when shrimp are not present may provide an acceptable means to control these baculoviruses. A temperature lower than 60°C for a period longer than 10 min might also be effective and would probably be more practical.

Insect viruses appear to be less tolerant of uv radiation than shrimp viruses. Jacques (1967) demonstrated complete inactivation of *T. ni* virus after a 1-min exposure to wavelengths of approximately 254 nm when 15 cm from the light source. Gudauskas and Canerday (1968) lowered the infectivity of NPV to *T. ni* by exposing the virus for 4–10 min 5–15 cm (2–6 inches) from the source, and they completely inactivated suspensions of *Heliothis* NPV with 5-min exposures 5 cm (2 inches) from the source. In contrast, our experiment required greater cumulative uv dosages to inactivate *B. penaei*. This difference in required dosages can be explained by the presence of *B. penaei* in a suspension. A layer of artificial sea water 10 cm deep absorbs 11 times more uv light (< 360 nm) than does pure water, and this absorption increases with turbidity (slightly turbid in the *B. penaei* suspension) and salt concentration (Riley and Skirrow, 1975). Momoyama (l989a), when he inactivated BMN virus suspended in sea water, used a wavelength of 254 nm at a distance of 30 cm from the light source (cumulative dosage: 4.1×10^5 $\mu W \cdot sec/cm^2$). The high cumulative uv dosages necessary to inactivate the tested aquatic baculoviruses may have been a result of the combination water, NaCl, and organic matter found in marine environments and not because of the structural or biochemical differences between shrimp and insect baculoviruses.

We believe our experimental design for the uv light study accurately reflects conditions found in aquaculture facilities, and we question the effectiveness of uv water sterilizers inactivating baculoviruses in these systems. Typical specifications for commercially made uv water-sterilizers include maximum flow rates that range from about 57,000 to 114,000 liters (l,500 to 30,000 gallons) per hour and use lamps emitting wavelengths of 254 nm. Our data indicate that high uv dosages generated 254-nm lamps can inactivate *B. penaeid*; however, even at low flow rates, contaminated water is in contact with the uv source for an insufficient period. The linear decrease in the prevalence of *B. penaei* infections in larvae observed 44 hr after exposure to virus treated with cumulative uv dosages of 1.77 and 3.54×10^6 $\mu W \cdot sec/cm^2$, as well as the resulting increase of infections to 100% of the larvae in these same groups by 72 hr postinfection, probably also reflects the selective inactivation

of some nonoccluded virions or gradual reduction in the number of infective virions. Decreasing the flow rates to levels low enough to completely inactivate both occluded and free virions (exposure for at least 30 min) would be impractical and not economical.

Presently, granular chlorine (HTH, active ingredient: calcium hypochlorite) is routinely used to disinfect many contaminated shrimp culture facilities, but this method requires extensive labor, time, and expense. Alternative methods of disinfection are continually being sought. Sodium hydroxide has recently been reported to be effective as a preventative for spread of *B. penaei* in Ecuador (Akamine and Moores, 1989). Our data, the first on the tolerance of *B. penaei* to selected chemical and physical conditions under controlled conditions, indicate that of the methods tested, thorough desiccation of equipment for at least 48 hr appears to be the most practical means of controlling or eliminating the agent. To investigate the phylogenetic relationship between infections in shrimps and insects, we encourage future studies comparing the effects of chemical and physical conditions of the infectivity of baculoviruses in natural environments and the physiological conditions necessary to release occluded virions infective to arthropod cells. Such studies should help explain the origin and evolution of these complex viruses.

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